Listing of Claims:

This listing of claims will replace all prior versions, and listings of claims in the application. No amendments have been made to the claims.

1. (Original) A method of preparing a plurality of chemical mixtures from a set number of inlets, the method comprising:

metering a volume of a first chemical from a first inlet to a microfluidic mixing structure;

metering a volume of a second chemical from a second inlet to the microfluidic mixing structure to create a first mixture comprising the first and second chemicals;

flowing the first mixture out of the microfluidic mixing structure;

again metering the volume of the first chemical from the first inlet to the microfluidic mixing structure; and

metering a volume of a third chemical from a third inlet to the microfluidic mixing structure to create a second mixture comprising the first and the third chemicals.

2. (Original) The method of claim 1 further comprising observing a property of the first and second mixtures.

3. (Original) The method of claim 2 wherein a property of the first and second mixtures is observed in the microfluidic mixing structure.

4. (Original) The method of claim 2 further comprising:

flowing the first and second mixtures to respective storage locations, and observing a property of the first and second mixtures in the respective storage chambers.

5. (Original) The method of claim 2 wherein:

metering the first chemical comprises flowing a known concentration of a macromolecule solution;

metering the second chemical comprises flowing a first concentration of a crystallizing agent solution;

metering the third chemical solution comprises flowing a second concentration of the crystallizing agent solution; and

observing the property of the first and second mixture comprises observing formation of solid material.

6. (Original) The method of claim 2 wherein:

metering the first chemical comprises flowing a known concentration of a crystallizing agent solution;

metering the second chemical comprises flowing a first concentration of a macromolecule solution;

metering the third chemical solution comprises flowing a second concentration of the macromolecule solution; and

observing the property of the first and second mixture comprises observing formation of solid material.

7. (Original) The method of claim 2 wherein:

metering the first chemical comprises flowing a known concentration of a crystallizing agent solution;

metering the second chemical comprises flowing a first concentration of a buffer solution; and

metering the third chemical solution comprises flowing a second concentration of the buffer solution;

the method further comprising metering to the first and second mixtures, a volume of a macromolecule solution, and observing formation of solid material in the first and second mixtures.

8. (Original) The method of claim 2 wherein:

metering the first chemical comprises flowing a known concentration of a macromolecule solution;

metering the second chemical comprises flowing a first concentration of a buffer solution; and

metering the third chemical solution comprises flowing a second concentration of the buffer solution;

the method further comprising metering to the first and second mixtures, a volume of a crystallizing agent solution, and observing formation of solid material in the first and second mixtures.

9. (Original) The method of claim 1 wherein at least one of metering the first and second chemicals comprises metering by peristaltic pumping action of an elastomer membrane deflected into a microfluidic channel containing one of the first and second chemicals.

10. (Original) The method of claim 1 wherein metering of the second and third chemicals comprises actuation of an elastomer membrane into a microfluidic flow channel by operation of a multiplexer.

11. (Original) The method of 1 in which the volume of each of the first and second mixtures comprises about 20 nL or less.

12. (Original) The method of 1 further comprising repeatedly preparing additional mixtures in the mixing structure to systematically characterize a chemical or biological response as a function of a composition of the mixture.

13. (Original) The method of 12 wherein macromolecule solubility is characterized.

14. (Original) The method of claim 13 wherein the first chemical comprises a membrane protein and the second chemical comprises a corresponding detectable ligand, the

mixing permitting determining solubility of the membrane protein folded in a shape as found in the cell membrane.

15. (Original) The method of claim 13 wherein mixing the first and second chemicals, and mixing the first and third chemicals, permits determination of a solubility limit intermediate between a soluble condition and a precipitation condition, the method further comprising:

performing batch crystallization experiments having conditions near the solubility limit.

6. (Original) The method of claim 15 wherein conditions of the batch crystallization experiments are within about ±50% of the immediate supersaturation (ISS) condition defined by:

$$ISS = \frac{PC\text{-}IMC}{IMC}, \text{ where:}$$

PC = macromolecule concentration; and

IMC = maximum macromolecule concentration that fails to result in solid formation within 1 minute or less from first exposing the macromolecule to the crystallizing agent..

17. (Original) The method of claim 15 wherein conditions of the batch screening macromolecule crystallization experiments are achieved by free interface diffusion between a first mixture comprising buffer mixed with a macromolecule, and a second mixture comprising buffer mixed with a crystallizing agent.

18. (Original) The method of claim 15 wherein the batch screening macromolecule crystallization experiments comprise fifty or more mixtures consuming 10 μL or less of a macromolecule solution.

19. (Original) The method of 15 in which characterization of macromolecule solubility and the batch screening experiments are performed on a single microfluidic device.

20. (Original) The method of 15 in which characterization of macromolecule solubility and batch screening experiments are performed on different microfluidic devices.

21. (Original) An apparatus comprising:

a microfluidic flow channel network formed in a first elastomer layer, the microfluidic flow channel network comprising a first set of inlet branches in fluid communication with a junction and with a reagent source, a second set of inlet branches in fluid communication with the junction and with a buffer source, and a mixing structure in fluid communication with the junction and with an outlet;

a first control channel network formed in a second elastomer layer adjacent to the first elastomer layer, the first control channel network adjacent to the first inlet branch set to define a first multiplexer structure configured to flow a select reagent into the junction; and

a second control channel network formed in the second elastomer layer, the second control channel network adjacent to the second inlet branch set to define a second multiplexer structure configured to flow a select buffer into the junction.

22. (Original) The apparatus of claim 21 wherein the junction comprises a second flow channel intersecting a first flow channel at first and second points separated by a distance.

23.. (Original) The apparatus of claim 22 wherein the first flow channel is branched along the distance.

24. (Original) The apparatus of claim 21 wherein the mixing structure comprises a closed circuit configured to be isolated from the junction and the outlet.

25. (Original) The apparatus of claim 21 wherein the mixing structure comprises a substantially circular shape.

26. (Original) The apparatus of claim 21 wherein the flow channel network further comprises an injector channel in fluid communication with the mixing structure.

27. (Original) The apparatus of claim 21 wherein the second elastomer layer defines at least three control channels overlying the flow channel network to define a peristaltic pumping structure configured to flow fluid through one of the first inlet branch set, the second inlet branch set, and the closed circuit.

28. (Original) The apparatus of claim 21 further comprising a sample storage structure in fluid communication with the outlet and configured to retain a sample from the mixing structure.

29. (Original) The apparatus of claim 28 wherein the sample storage structure comprises an elongated flow channel.

30. (Original) The apparatus of claim 29 wherein the elongated flow channel is dead-ended.

31. (Original) The apparatus of claim 30 wherein an end of the elongated flow channel opposite the inlet is gated by a valve.

32. (Original) The apparatus of claim 31 further comprising a multiplexer structure governing fluidic access to the elongated flow channel.

33. (Original) The apparatus of claim 29 wherein the storage structure comprises an array of storage vessels connected by rows and columns of flow channels.

34. (Original) The apparatus of claim 33 wherein the array of storage vessels comprises paired vessels in fluid communication through a valved connecting channel.

35. (Original) A method of identifying conditions conducive to crystallization comprising:

preparing a first solution including a solvent and a sample at a first concentration;

mixing with the first solution, a second solution including a crystallizing agent;

noting a first condition at which solid phase first appears in the mixture;

adding additional solvent to the mixture;

noting a second condition at which solid phase disappears in the mixture; and

identifying a hysteresis between the first and second conditions.

36. (Original) The method of claim 35 wherein the first and second conditions define a concentration of sample and crystallizing agent.

37. (Original) The method of claim 35 wherein:

the crystallizing agent is introduced into a closed circuit microfluidic channel containing the sample; and

the solvent is then introduced into the closed circuit microfluidic channel.

38. (Original) The method of claim 37 wherein at least one of the crystallizing agent and the solvent are introduced by a pumping action of an elastomer membrane.

39. (Original) The method of claim 35 wherein the first and second conditions are determined by optical interrogation of a microfluidic flow channel containing the sample and the crystallizing agent.

40. (Original) The method of claim 35 wherein a microfluidic structure microfabricated from elastomer is optically interrogated.

41. (Original) A method of identifying conditions conducive to crystallization comprising:

detecting scattering of light as a crystallizing agent is mixed with a solution containing a sample;

correlating the detected scattered light with a known range of a second virial coefficient characteristic of a protein crystal-containing solution.

42. (Original) The method of claim 41 wherein scattering is detected as the crystallizing agent is flowed into a closed circuit microfluidic channel containing the sample.

43. (Original) The method of claim 41 wherein the crystallizing agent is pumped into the closed circuit microfluidic channel by actuation of an elastomer membrane.

44. (Original) A method of controlling flow through a microfluidic device comprising:

disposing a fluid in a flow channel;

applying a pressure to a control channel adjacent to and separated from the flow channel to cause an intervening elastomer membrane to deflect into the flow channel; and

maintaining a baseline pressure of the fluid in the flow channel at greater than 5 psig while relaxing the pressure applied to the control channel to bias the elastomer membrane out of the flow channel.

45. (Original) The method of claim 44 wherein pressure is applied to the control channel in the form of a flux of a volume of air, such that the elevated pressure maintained within the flow channel reduces formation of air bubbles in the flow channel.

46. (Original) The method of claim 44 wherein the elastomer membrane may be actuated into and out of the flow channel at a frequency of 50 Hz or greater.

47. (Original) The method of claim 44 wherein the flow channel comprises a closed circuit, and actuation of the elastomer membrane may cause fluid to flow through the closed circuit at a frequency of about 4 Hz.

48. (Original) A method of identifying conditions conducive to solubilization of a membrane protein, the method comprising:

introducing a solid membrane protein into a microfluidic closed circuit mixing structure;

introducing an amphiphilic moiety into the microfluidic closed circuit mixing structure;

exposing a mixture of the membrane protein and the amphiphilic moiety to a detectable ligand configured to bind only with a form of the membrane protein as folded in a membrane; and

detecting the ligand to identify the folded form of the membrane protein in solution.

49. (Original) The method of claim 48 wherein the mixture is exposed to the detectable ligand within the microfluidic closed circuit mixing structure.

50. (Original) The method of claim 48 wherein the mixture is exposed to the detectable ligand in a microfluidic flow channel receiving a flow from the microfluidic closed circuit mixing structure.

51. (Original) The method of claim 50 wherein:

the protein is tagged prior to exposure to the ligand; and

the soluble tagged membrane protein binds to a surface within the microfluidic flow channel.